Application No. 10/609,346
Response Dated November 21, 2006
Reply to Office Action of August 24, 2006

Amendment to the Specification:

1. On page 28, please amend the last paragraph to:

In the present invention, the albumin's plasma transporter function and the therapeutic function of the CPSF are integrated into a fusion form. The presence of albumin may confer a superior stability to the CPSF by resisting degradation by proteases in the blood circulation, thus significantly prolonging the plasma half life of the CPSF. Due to the masking effect of a bulky albumin, a combination of different CPSFs fused with albumin ~~in the combination~~ may impose less interference with the biological function(s) among different albumin fused CPSFs ~~of each other~~ than among a combination of the "naked" CPSFs. Further, a CPSF fused with albumin may be slowly released in the system over an extensive period of time, thereby reducing the toxicity associated with injection of the CPSF alone in abnormally high concentrations in the body. Such a slow release mode of action of the fusion protein combination can significantly reduce the amount and/or frequency of injections of the CPSF, thereby further reducing the side effects of CPSFs. Such combinations are particularly useful for stimulating multiple blood cell proliferation after or before the chemo- or radiation therapy of cancer patients ~~who are~~ whose tolerance for frequent, high dose injection of CPSF are seriously compromised.

2. On page 29, please amend the last paragraph to:

It is also known that "naked" cytokines (i.e., cytokines not fused to another protein such as HSA) are quite unstable when stored and have a short plasma half-life. Clearly, a therapeutic protein with such a weak stability in vivo constitutes a major handicap. In effect, repeated injections of the product, which are costly and inconvenient for patient, or an administration of product by perfusion, become necessary to attain an efficient concentration in plasma. Due to its extended plasma

half-life and enhanced stability, the HSA/CPSF fusion proteins of the present invention and their combinations, e.g., HSA fusions with hIL-11, hEPO, hG-CSF and hGM-CSF, can be used to stimulate the production of multiple blood cells in plasma of humans.

3. On page 38, please amend the last paragraph to:

Five µg of each linear plasmid DNA was used to transform 80 µl of the freshly made yeast cells in an ice-cold 0.2 cm electroporation cuvette. The cells mixed with plasmid DNA were pulsed for 5-10 ms with field strength of 7500V/cm. After the pulse, 1 ml of ice-cold 1 M sorbitol was immediately added into the cuvette and the content was transferred to a sterile 15 ml tube. The transformed cells were incubated ~~in~~ at 30° C. without shaking for 2 hours then spread on pre-made YPD-agar plates with 100 g/ml Zeocin. The colonies were identified with the insert and the expression level was determined by SDS-PAGE or western-blot with proper antibodies.

4. On page 39, please amend the last paragraph to:

Mouse monoclonal anti-human serum albumin (Sigma) was used for immunoblotting on a SDS-PAGE gel. A typical Western blot experiment was carried on by electrophoresis transfer the protein from SDS-PAG to a nylon or nitrocellulose filter and incubated with a specific antibody (as the "first antibody"). Then an anti-first antibody ~~would add to binding on~~ was added to bind to the first antibody (as the "second antibody"). The second antibody was labeled with Fluorescence and the filter was exposed to an X-ray film. Protein molecular weight standard was used to determine the protein size. The results (FIG. 3) showed that the expressed recombinant proteins, HSA, HSA-CPSF therapeutic fusion protein, had an expected molecular weight and also had the same antigen as that of HSA prepared from a human blood plasma (Sigma). Using monoclonal anti-hIL-11 specific antibody as the first antibody, the HSA/hIL-11 fusion protein and human IL-11 (R&D System) had the

same antigen and showed that the molar ratio of HSA to hIL-11 in the HAS/hIL-11 fusion protein is as expected (FIG. 4). Using monoclonal anti-hGMCSF specific antibody (R&D System) as the first antibody, the HSA-GMCSF fusion protein and human GMCSF (R&D System) had the same antigen and showed that the molar ratio of HSA to GMCSF in the HAS/GMCSF fusion protein is as expected (FIG. 5).

5. On page 42, please amend the third paragraph to:

The results showed that the bioactivity of hEPO fused to HSA had {fraction (1/10)} of activity compared with the standard (FIG. 7). The size of HSA-EPO fusion protein molecule may be too large, which prevents the anti-EPO antibody from efficiently binding to the EPO molecule fused to ~~HAS~~ HSA, thereby reducing the sensitivity of the detection in this bioassay.

6. On page 42, please amend the last paragraph to:

Using HSA/hIL-11 as an example, the stability of this HSA-CPSF fusion protein was tested at different time points at 37° C. and 50° C. 5ng of human IL-11 from bacteria or 5 ng of rHSA/hIL-11 was put into 200 µl thin-well PCR tube with 200 µl of tissue culture medium RPM1 without fetal bovine serum and other components. The tubes were sealed and left in water ~~both~~ bath. Samples were taken out at different time points and immediately put into -80° C. for storage. After all of samples were collected, a cell proliferation test on T1165 cell line was carried out by incorporating ^{3}H-Thymidine into newly synthesized DNA of proliferating cells. The control of the test was set up in the same way as that in the bioassay of human IL-11 (See Paul et al., 1990). As shown in FIG. 8, after 5 weeks in 37° C. (Panel A), the bioactivity of ~~HAS/IL-11~~ HSA/IL-11 still remained the same, but the "naked" human IL-11 lost almost all of its bioactivity after three weeks at 37° C. At 50° C. (Panel B), the "naked" human IL-11 lost its the bioactivity completely in 2 weeks. And the ~~HAS/IL-11~~ HSA/IL-11 fusion

protein still retained at least half of its bioactivity. These results indicate that a CPSF fused to human albumin can have a longer storage time and more resistant to degradation in harsh environment such as high temperatures.

7. On page 43, please amend the second paragraph to:

Rabbits (2.3-2.6 Kg) were injected with 200 µl of recombinant proteins prepared above at day 1, day 3 and day 6. Rabbit A was injected with a mixture of 150U/kg EPO (about 10 µg of protein) and 100 µg recombinant HSA (rHSA); Rabbit B with a mixture of 120 µg IL-11 and 100 µg rHSA; and Rabbit C with a mixture of 120 µg rHSA/hIL-11 fusion (equivalent of 50 µg of pure bacteria-expressed IL-11), 27 µg rHSA/hEPO fusion (equivalent of 150 U of HSA-EPO determined by using the EPO-ELISA Kit, R&D System Inc.) and 50 µg rHSA. Rabbit D was injected with 120 ~~82 g~~ µg of HSA-IL-11 and about 80 µg rHSA.

8. On page 43, please amend the third paragraph to:

Blood samples were collected and ~~blood red cells~~ red blood cell and platelet numbers were counted by a hemacytometer. The results are shown in FIG. 9 (panels A, B, and C). The cell counts on the starting day of the experiment was treated as the base line. After the treatment with the proteins, the cell counts were compared with those on the starting day and the changes were plotted in the graphs in FIG. 9.

9. On page 43, please amend the last paragraph to:

As shown in panel A of FIG. 9, both EPO and ~~HAS/EPO~~ HSA/EPO fusion protein stimulated the production of erythrocytes in rabbit A and C, respectively. However, in rabbit A injected with the naked EPO, the level of erythrocytes reached a peak around day 35 post first injection and then declined quickly to reach near a

baseline level around day 55. In contrast, in rabbit C injected with ~~HAS/EPO~~ HSA/EPO fusion protein, the level of erythrocytes increased and reached a plateau around day 35 post first injection but remained high till the end of the experiment. These results demonstrated that ~~HAS/EPO~~ HSA/EPO fusion protein has a much longer plasma half-life than the naked EPO and remains bioactive for a much longer time than the naked EPO in vivo. As also shown in this panel, IL-11 had little effect in stimulation of erythrocyte production in rabbit B.

10. On page 44, please amend the second paragraph to:

As shown in panel B of FIG. 9, both IL-11 and ~~HAS/IL-11~~ HSA/IL-11 fusion protein stimulated the production of platelets in rabbit B and C, respectively. However, in rabbit B injected with the naked IL-11, the level of platelets reached a peak around day 20 post first injection and then declined quickly to reach near a baseline level around day 43. In contrast, in rabbit C injected with HSA/IL11 fusion protein, the level of platelets increased and reached a plateau around day 40 post first injection but remained high till the end of the experiment. These results demonstrated that HSA/IL-11 fusion protein has a much longer plasma half-life than the naked IL-11 and remains bioactive for a much longer time than the naked IL-11 in vivo. As also shown in this panel, EPO had little effect in stimulation of platelet production in rabbit A.